during different stages of lymphoid and myeloid lineage differentiation. The results identified enhancers F, H, and I as potent stimulators of transcription, but only in permissive chromatin, thus acting similarly to classical enhancers that promote initiation and elongation of transcription. In contrast, enhancers D and J were efficient in counteracting repressive chromatin, which likely is the result of recruitment of epigenetic factors that promote a transcriptionally permissive chromatin state to that region.

All of the *Ikzf1* enhancers were able to stimulate the *Ikzf1* promoter-based expression in B and myeloid cells. However, only 2 enhancers (D and H) were able to stimulate transcription in T cells as well. The D enhancer was the only one capable of stimulating GFP expression above the basal level in the LMPP that marks the earliest stage of lymphoid differentiation. Further analysis revealed that enhancer D is critical in counteracting repressive chromatin at the *Ikzf1* locus and for maintaining a high level of transcription, and these functions could not be compensated by any other *Ikzf1* enhancers. The authors further dissected the enhancer D and identified subdomains that confer stage-specific expression in T-lineage cells.

Although individual enhancers were capable of stimulating *Ikzf1* expression, their activity could not replicate the activity of the wild-type endogenous *Ikzf1* locus. The endogenous gene expression pattern in hematopoietic cells and in the neuronal lineage was ensured when 9 of the 10 conserved *Ikzf1*enhancers were combined in a miniregulatory locus.

The authors conclude these studies by analyzing previous ChIP-SEQ data to identify a network of transcription factors that bind in vivo at the *Ikzf1* enhancers. These analyses revealed binding of HEB, runt-related transcription factor 1 (Runx1), T-cell factor 1, and Ikaros in thymocytes; avian myelocytomatosis oncogene (c-Myc) and avian erythroblastosis virus E26 oncogene 1 (Ets-1) in B cells; and GATA binding protein 1 (GATA1), GATA2, and stem cell leukemia/T cell acute lymphocytic leukemia 1 (SCL/Tal1) in erythroid precursors. In addition, motif search for transcription binding sites at enhancers D and H identified enrichment of binding sites for several transcriptional factors with important roles in hematopoiesis; eg, Runx, Homeobox

A9 (HoxA9), special AT-rich sequence binding protein 1 (Satb1), Interferon regulatory factor-1 (Irf1), Irf4, CCAAT/enhancer binding protein–α (C/EBP-α), C/EBP-β, myocyte enhancer factor 2C (MEF2C), and E2A. The proposed working model by Yoshida et al, based on these findings, is outlined (see figure).

What are some of the implications? Next-generation sequencing has identified inactivating deletions and mutations at the *Ikzf1* locus in a large subset of B-cell precursor acute lymphoblastic leukemia (B-ALL) and in early T-cell ALL in humans. These genetic alterations that result in reduced *Ikzf1* activity are poor prognostic indicators in pre-B-ALL. The identification of *Ikzf1* enhancers that are essential for optimal *Ikzf1* expression provides an additional tool to identify potential prognostic markers. The obvious next step would include sequencing *Ikzf1* enhancer elements and correlation of potential mutations and/or polymorphism in these regions with the development and/or outcome of leukemia. With the rapid development of next-generation sequencing technology and the decreased cost of sequencing, these assays are quite feasible and may yield important diagnostic information.

The identification of a network of transcription factors that positively regulates *Ikzf1* expression provides an opportunity to uncover larger signaling pathways that control normal and malignant hematopoiesis. Besides the obvious impact on scientific advances in the field, this could also have important therapeutic implications. The modulation

of signaling pathways that control *Ikzf1* expression could be a powerful tool for the treatment of hematopoietic malignancies and some immunological disorders. This story is just developing.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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● ● ● **CLINICAL TRIALS & OBSERVATIONS**

Comment on Wang et al, page 3122

# Can CRd be a standard for refractory myeloma?

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In this issue of *Blood*, Wang et al describe that the combination of carfilzomib, lenalidomide, and low–dose dexamethasone (CRd) can be a valuable option in relapsed and refractory multiple myeloma patients.[1]

In 1958, Blokhin et al reported the first experience with sarcolysin (melphalan) in neoplastic diseases,[2] and a few years

later Drs D. Bergsagel and R. Alexanian pioneered studies showing the efficacy of melphalan in multiple myeloma. However,

in the subsequent 30 to 40 years, progress in myeloma treatment remained stagnant. In fact, the major innovation was the use of high-dose therapy, which was again mainly based on melphalan, followed by autologous stem cell support. The situation has significantly changed in the last decade with the approval of 2 immunomodulatory drugs (IMIDs) (thalidomide and lenalidomide) and 1 proteasome inhibitor (PI) (bortezomib).

Carfilzomib is a second-generation PI that belongs to the epoxiketone family and irreversibly binds the chymotrypsin-like activity of the proteasome. It has shown marked activity, as simple agent, in phase 1 and 2 clinical trials, with 40% to 52% of responses in bortezomib-naïve patients and 17% to 19% in bortezomib-refractory cases and a very low incidence of peripheral neuropathy (PN).[3-5] The possibility of combining PI with IMIDs is very attractive, and the positive results of the bortezomib-lenalidomide-dexamethasone (VRD) combination[6] were the basis for the study reported in this issue of *Blood* by Wang et al.[1] In a previous phase 1b dose-escalation study,[7] the same authors identified the maximum planned dose (MPD) for CRd as $20/27$ mg/m$^2$ for carfilzomib, 25 mg for lenalidomide, and 40 mg for dexamethasone. Here, they show the efficacy and safety of CRd at the MPD in a total of 52 patients; the response rate (RR) was 76.9%, with a median progression-free survival (PFS) of 15.4 months. The benefit of adding carfilzomib to lenalidomide-dexamethasone will be determined in the ASPIRE randomized trial that compares CRd vs Lenalidomide plus low-dose dexamethasone, but the present data already suggest that in lenalidomide-naïve patients, the RR (85%) and median PFS (not reached) is superior to that previously reported for lenalidomide-dexamethasone.[8,9] Moreover, 68% of patients refractory to lenalidomide responded to CRd with a median PFS of 9.9 months. Whether or not the results with CRd combination are superior to previously reported data with VRD is difficult to determine, because the patient populations were rather heterogeneous and small in size. Perhaps the clearest advantage of CRd is the lower incidence of PN (27% vs 64% for any grade PN); however, in the Richardson trial, the

more friendly bortezomib schedule (weekly and subcutaneous) was not used. Accordingly, the answer to this question will only come from a randomized trial.

The second relevant question, in the relapse setting, is whether it is preferable to use a combination of the 2 new drugs (PI plus IMID) or to combine one of them with an alkylator (ie, cyclophosphamide) and to reserve the other one for subsequent relapses. In this comparison, costs should also be taken into consideration. How many countries will pay for this expensive triple combination unless there is a study design showing that the triplet at relapse is superior in terms of overall survival (not in terms of RR or PFS) to a sequential treatment approach? If this proves to be positive, then CRd will be cost-effective and will become a new standard for relapse/refractory patients.

A different scenario at relapse is that of young patients who are candidates to receive a transplant as part of the rescue therapy, particularly if this is an allotransplant, because in this setting we want to obtain the best possible response as soon as possible, and therefore the combination of a PI with IMIDs is clearly justified.

*Conflict-of-interest disclosure: The author has participated in some advisory boards for Millennium, Celgene, Novartis, Onyx, and Janssen.* ■

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● ● ● **IMMUNOBIOLOGY**

Comment on Albanesi et al, page 3160

# Neutrophils: "neu players" in antibody therapy?

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In this issue of *Blood*, Albanesi et al have added weight to the contention that neutrophils are an important effector population in monoclonal antibody (mAb)-mediated tumor cell clearance. Their data, obtained using subcutaneous tumor models and an extensive panel of genetically modified mice, demonstrate that neutrophils are required for mAb efficacy and that they do so through a Syk-dependent Fcγ receptor (FcγR)–mediated mechanism.[1]

Antibody therapeutics which target tumor cells, directly recruiting natural effectors, have become a mainstay for managing hematologic malignancies, with the anti-CD20 mAb rituximab heralding a new era in lymphoma treatment. In contrast, the usefulness of mAbs against solid tumors has been limited and largely confined to reagents, such as anti-her2/neu, anti–epidermal growth factor receptor, and